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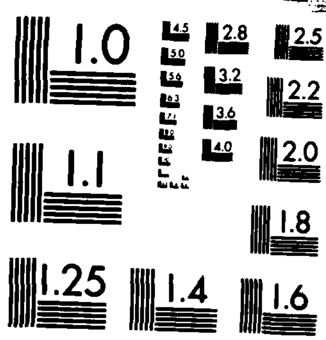

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LONG-TERM EFFECTS OF DREDGING OPERATIONS PROGRAM

MISCELLANEOUS PAPER D-84-1

EVALUATION OF THREE FISH SPECIES AS BIOASSAY ORGANISMS FOR DREDGED MATERIAL TESTING

by

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Three fish species, Cyprinodon variegatus, Fundulus similis, and Menidia menidia, were evaluated to determine which is most suitable as a bioassay organism for solid phase testing of dredged material. Acute toxicity and bioaccumulation of polychlorinated biphenyls (PCBs) were monitored for 52 days of exposure to two types of dredged material collected from New York Harbor. Cyprinodon variegatus displayed the most consistent accumulation of PCBs.

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However, no definitive statement can be made regarding acute toxicity response because of poor control survival. Review of the literature indicates that *M. mendidia* is the most sensitive of the three species examined and, consequently, it is felt that this species should be strongly considered as a candidate for solid phase testing.

Preface

This study was conducted by the U. S. Environmental Protection Agency, Environmental Research Laboratory, Gulf Breeze, Fla. (ERLGB). Financial sponsorship was primarily from the U. S. Army Engineer District, New York, through the Environmental Laboratory (EL) of the U. S. Army Engineer Waterways Experiment Station (WES), Vicksburg, Miss. The WES also contributed support under the Long-Term Effects of Dredging Operations (LEDO) Program, which is sponsored by the Office, Chief of Engineers (OCE), U. S. Army.

The author gratefully acknowledges the assistance of ERLGB personnel: Ms. T. Dunn for technical assistance, Dr. C. Deans for programming and statistical assistance, Mr. S. Foss for illustrations, Mrs. B. Jackson for editing, and Mrs. M. Stubbs for typing. The study was conducted under the general supervision of Dr. Al Bourquin, Chief, Processes and Effects Branch. The Director of ERLGB during this study was Dr. Henry Enos.

The WES project manager was Dr. R. Peddicord, under the general supervision of Dr. C. Lee, Chief, Contaminant Mobility and Regulatory Criteria Group; Mr. D. Robey, Chief, Ecosystem Research and Simulation Division; and Dr. J. Harrison, Chief, EL. This work was coordinated with other EL dredging studies by the Environmental Effects of Dredging Programs, Mr. C. Calhoun, Manager. The New York District project manager was Mr. J. Mansky. Technical Monitors for LEDO were Drs. J. Hall and W. Klesch, OCE, and Mr. C. Hummer, Water Resources Support Center.

Commander and Director of the WES during conduct of the study and preparation of the report was COL Tilford C. Creel, CE. Technical Director was Mr. F. R. Brown.

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EVALUATION OF THREE FISH SPECIES AS BIOASSAY ORGANISMS FOR DREDGED MATERIAL TESTING

Introduction

Background

1. The Marine Protection, Research and Sanctuaries Act of 1972 (P.L. 92-532) regulates the disposal of dredged material in the ocean. Implementing criteria published in the Federal Register of January 11, 1977, require that dredged material proposed for ocean disposal first be evaluated on the basis of biological tests to ensure that no unacceptable adverse effects are produced. Because field studies and monitoring can measure consequences of contaminant disposal only after the fact, the U.S. Environmental Protection Agency (EPA) and the U.S. Army Corps of Engineers (CE) recommend the use of laboratory bioassays (EPA/CE 1977) to provide the data required to assess the potential ecological impact resulting from ocean disposal operations.

2. Regulations governing dredged material disposal have focused on two primary measures of biological effects: acute toxicity and bioaccumulation potential of contaminants in representative aquatic organisms. The biological testing recommended by EPA/CE (1977) presently consists of 4-day acute toxicity tests conducted on the liquid and suspended phases and 10-day toxicity and bioaccumulation tests conducted on the solid phase of dredged material. The liquid and suspended particulate tests are designed primarily to predict impact on water column organisms, whereas solid phase tests are utilized to illucidate effects on benthic organisms.

3. A primary consideration in the design and implementation of bioassays is species selection. The efficacy of the bioassay results is directly

related to the relevancy of the species tested. Organisms selected for testing should be representative of indigenous biota at the disposal site at least in terms of phylogeny and sensitivity to toxicants. The present list of suggested species for solid-phase testing (EPA/CE 1977) consists entirely of infaunal and epifaunal invertebrates. In order to improve the applicability of bioassay data for dredged material evaluation, the array of test organisms should include a suitable fish species.

Objective

4. The objective of this study was to evaluate marine fish species in terms of their acute sensitivity to moderately contaminated sediments and their potential for bioaccumulation of polychlorinated biphenyl (PCB), a ubiquitous organic contaminant. It was the intention of this study to evaluate these organisms from the perspective of a contracting laboratory required to use standard methods and to apply recommended procedures for dredged material evaluation (EPA/CE 1977). We envisioned the use of a fish species as an alternative to test organisms currently used.

Considerations for bioassay species selection

5. A rationale to aid in selection of appropriate bioassay organisms was developed by Shuba, Petrocelli, and Bentley (1981). Their list of selection factors includes:

a. The organism is appropriately sensitive and is found at, or is related to, species at the disposal sites.

b. The organism is readily available throughout the year, either through field collection or purchasing.

c. A toxicological data base exists for the species, and response to the same toxicant is reproducible.

d. The organism can be maintained in a healthy condition in the laboratory.

e. The organism is culturable in the laboratory.

f. The organism occurs over a wide geographic area.

g. The organism is economically or ecologically important or both.

Additional factors that apply to species selected for bioaccumulation studies are: the organism should accumulate the pollutant without excessive mortality at concentrations found in the environment, and the organism should be of adequate size for tissue analysis (Phillips 1980).

6. Three fish species that meet the selection criteria are the sheepshead minnow (*Cyprinodon variegatus*), the killifish (*Fundulus similus*), and the Atlantic silverside (*Menidia menidia*). These species were selected for study and tested with dredged material taken from New York Harbor.

Methods and Materials

Organisms

7. This study was conducted at the EPA Environmental Research Laboratory, Gulf Breeze, Fla., from June through September 1982. *Menidia menidia* were purchased from a biological supply company and shipped via airfreight to Gulf Breeze. *Cyprinodon variegatus* and *F. similis* were collected from Santa Rosa Sound, Fla. All animals were acclimated to test conditions in the laboratory for at least 2 weeks prior to testing.

Sediments

8. Dredged material used in this study was collected from two sites in New York Harbor. Sediments (designated A and B) were analyzed for particle size, percentage moisture (EPA/CE 1981), organic content (by combustion at 500°C), and dry weight concentrations of PCBs (quantified as Aroclor^R 1254 and 1242).

Exposure system

9. The three test species were exposed to sediments, using the method of Rubinstein, Lores, and Gregory (1983) (Figure 1). Glass aquaria (37 ℓ) were used as test chambers. Three replicate aquaria were used for each species and sediment type. Control aquaria containing clean beach sand were also set up for each species. Seawater, pumped from Santa Rosa Sound and filtered through a 20-μm filter, was delivered to a headbox in the laboratory. Flow to each aquarium was maintained at 25 ± 1 ℓ/hr. Effluent water was routed through an in-line sediment trap to a holding pond. Temperature and salinity were monitored daily; flow rate, dissolved oxygen (DO), pH, and Eh were measured weekly. Temperature was maintained at 22 ± 1°C by water-chiller units (Mini-cool DI-100) mounted in the headbox. Salinity ranged from 26 to 32 °/oo during the test period.

10. Test sediments were sieved (2-mm mesh) to remove large debris and thoroughly mixed to ensure homogeneity. Aquaria received 3.5 ℓ of the appropriate sediment type, which formed a layer approximately 3.5 cm thick. Control aquaria received a 3.5-cm layer of washed beach sand. Tests on the three species were conducted in parallel; each aquaria received a single species. Loading in aquaria reflected size differences among species. Thirty *Menidia* (average weight ($\bar{x}$) = 1.6 g), 20 *Cyprinodon* ($\bar{x}$ = 3.0 g), and 10 *Fundulus* ($\bar{x}$ = 5.5 g) were placed in designated aquaria containing sediment A or B or control sand. Fish were fed a flake food (Tetra SM80, Tetra Werke West Germany) at a rate estimated to be 2 percent of body weight per day. Mortality and PCB accumulation were monitored for 52 days.

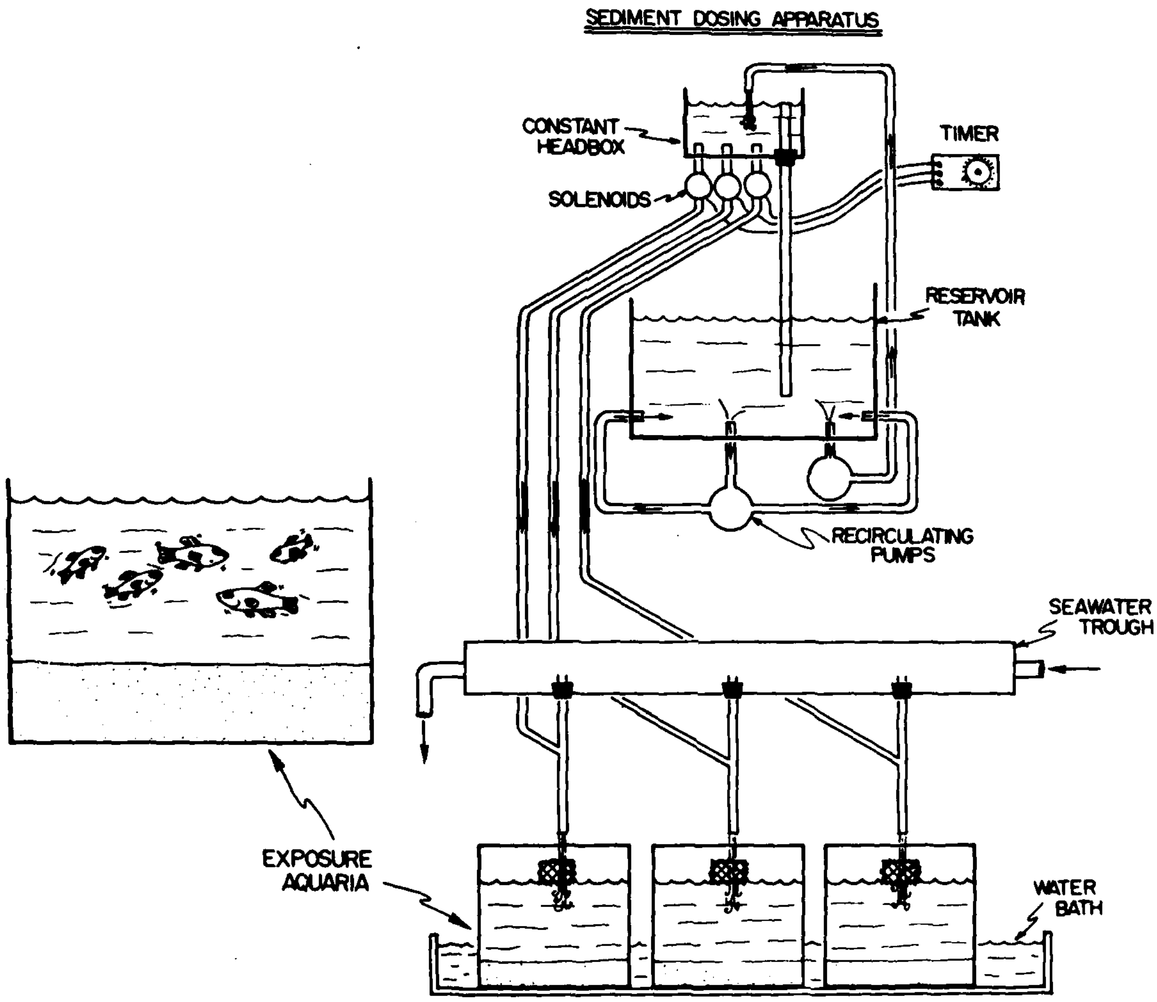

Figure 1. Flow-through exposure system and sediment resuspension apparatus (from Rubinstein, Lores, and Gregory 1983)

11. To provide sediment resuspensions in aquaria, we utilized the dosing apparatus of Rubinstein et al. (1980). For this study, a suspended solids load of 100 mg/l (dry weight) was delivered to aquaria containing sediments A and B at six hour intervals. Control aquaria received no suspended solids. Sediments remained in suspension for approximately 20 minutes before either settling or being slowly flushed out of the aquaria.

12. Mortality was monitored several times during the day and dead fish were removed immediately upon discovery. Percentage mortality was pooled for each species, plotted as a function of time, and compared with control mortality (SAS 1982). Background concentrations of PCBs in fish tissue and sediments were analyzed for PCB whole-body residues on days 4, 10, 17, 24, 38, and 52. Sediment samples (cores) were removed from aquaria and analyzed for PCBs on days 1, 10, and 52. Triplicate analyses (N = 3) were conducted for each species at each sampling interval. Fish were placed in uncontaminated flowing seawater for 24 hrs prior to tissue analysis to purge residual material from the intestinal tract.

Chemical analysis

13. Tissue samples (50 percent tissue in distilled water, w/w) of 1 to 8 g in 40-ml centrifuge tubes were homogenized in Polytron homogenizers (model PCU-2 Brinkman Instruments) and extracted three times with 10, 5, and 5 ml of acetonitrile for 15 to 30 sec. After each homgenization, the samples were centrifuged and the supernate decanted. Acetonitrile extracts (20 ml) were combined with 75 ml of 2 percent Na_2SO_4, then extracted three times with 10 ml hexane. The samples were shaken manually for 1 min and the phases allowed to separate (any emulsions were broken by

sonicating the samples). The hexane layer was transfered to 40-ml centrifuge tubes and concentrated using a gentle stream of nitrogen to reduce the volume to 1 to 2 ml. The concentrate was then transferred to a Florisil column to cleanup.

14. PCBs were extracted from sediments by the Soxhlet method of Bellar, Lichtenberg, and Lonneman (1980). Extracts were treated with mercury to remove sulfur and transferred to a Florisil column to remove other organic contaminants.

15. Kontes Chromoflex columns (9 mm) with a small glass wool plug in the tip were packed with 4 g of Florisil and topped with about 25 mm of anhydrous Na_2SO_4. Florisil, Na_2SO_4, and glass wool were dried previously and stored in an oven at 130°C. The Florisil column was packed just before use, allowed to cool, and eluted with 10 ml of hexane. When the hexane reached the top of the Na_2SO_4, sample extracts were layered on the column along with two 0.5-ml hexane rinses of the sample container. The PCBs were then eluted with 15 ml of hexane, followed by 10 ml 1 percent methanol in hexane. The eluate was concentrated to 1.0 ml and analyzed by gas chromatography.

16. The sediments contained considerable amounts of sulfur which can interfere with the chromatography of early eluting peaks. After the sample had been passed through Florisil and concentrated to a volume of 1.0 to 5.0 ml, sulfur was removed from all sediment sample extracts by addition of 0.2 to 1 ml of elemental mercury. The samples were shaken until all the sulfur had reacted and were analyzed by gas chromatography.

17. Gas chromatography was carried out on a Hewlett-Packard 5710 gas chromatograph with linear electron-capture detector operated at 300°C and a 1.8-m glass column (4 mm ID x 6 mm OD) packed with 3 percent OV-101

on 80/100 mesh-Supelcoport maintained at 200°C for Aroclor 1242 and at 220°C for Aroclor 1254. The carrier gas was 10 percent argon at a flow rate of 60 ml/min.

18. PCB quantification was done by the method of Webb and McCall (1973). The reference standard, obtained from EPA Analytical Standards Branch, Cincinnati, Ohio, was described by Sawyer (1978). Only Aroclor 1242 and 1254 isomers were quantified. Recoveries from spiked samples averaged 75 percent. Values presented in this report were not corrected for percentage recovery.

Results and Discussion

19. Particle size, PCB concentration (dry weight), percentage moisture, and percentage organics for sediments A and B are summarized below:

Table 1. Characterization of Test Sediments Collected from Two Sites in New York Harbor

Sediment	Sand-silt-clay percent	Organics percent	Moisture percent	PCBs µg/g dry wt.
A	28-62-10	13	70	6.90
B	27-55-18	7	55	0.31

PCB concentrations in both sediments appeared to diminish over time (Fig. 2). Although a small amount of suspended sediment was continuously pulsed into exposure aquaria, a net loss of sediment was observed upon termination of the test. Initial dry weight PCB concentration averaged 6.90 μg/g for sediment A and 0.33 μg/g for sediment B (N = 3). After 52 days, PCB concentrations measured 4.22 μg/g for sediment A and 0.13 μg/g for sediment B.

Mortality

20. During the first several weeks of this study, M. menidia and C. variegatus exposed to test and control sediments displayed high mortality (Fig. 3). This observation is not consistent with previous findings which indicate low order toxicity from dredged material (i.e. Suszkowski and Mansky 1982; Rubinstein et al. 1983). A possible explanation, although not completely satisfactory, is the extremely high turbidity (due to the swimming activity of fish) observed in exposure aquaria during the early days of the study. In time, resuspension diminished as sediments became more consolidated. However, the high mortality (60 percent) observed in control Menidia cannot be explained by high tubidity. In this case, death probably resulted from shipping and handling stress. Initial attempts to ship adult Menidia resulted in excessive mortality upon arrival at Gulf Breeze. The loading density of fish in subsequent shipments was reduced, which resulted in healthier organisms. Even so, some mortality still occurred during acclimation, but no deaths were observed for several days prior to the start of the study. Possibly, handling during initiation of the study contributed further to the stressed condition of the animals, resulting in the mortality observed in M. menidia during the first weeks of exposure.

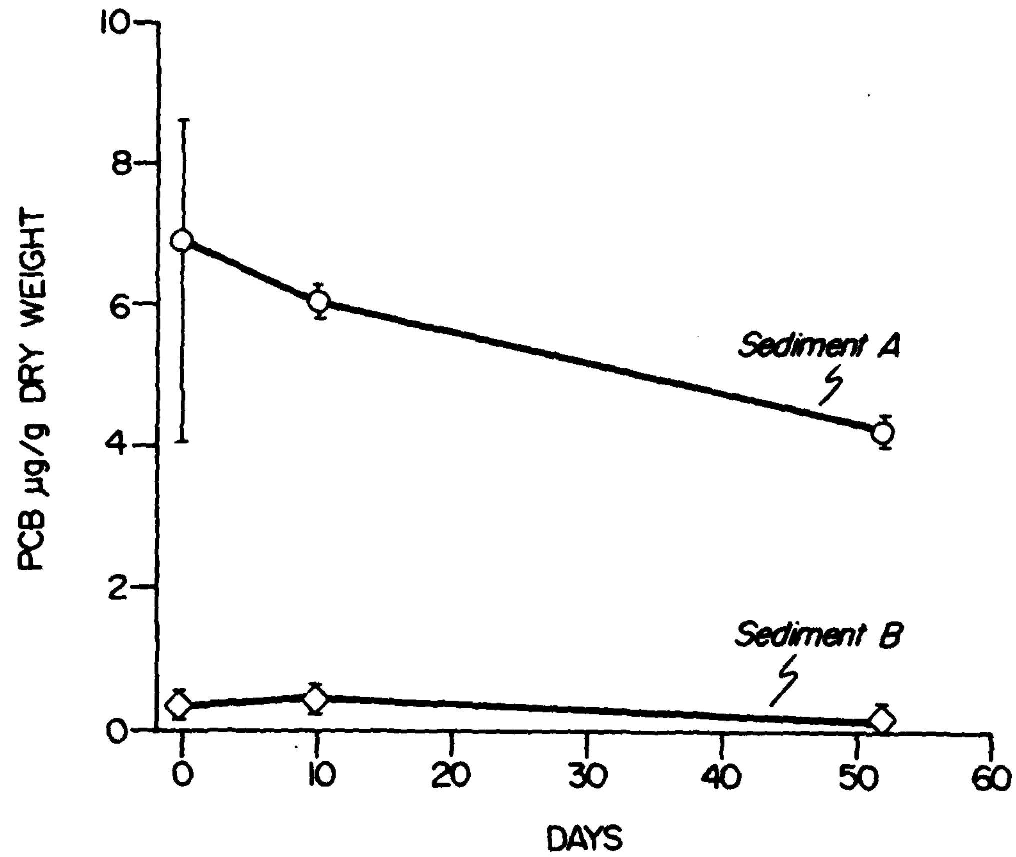

Figure 2. PCB concentration for sediments A and B during the 52-day exposure period (N = 3)

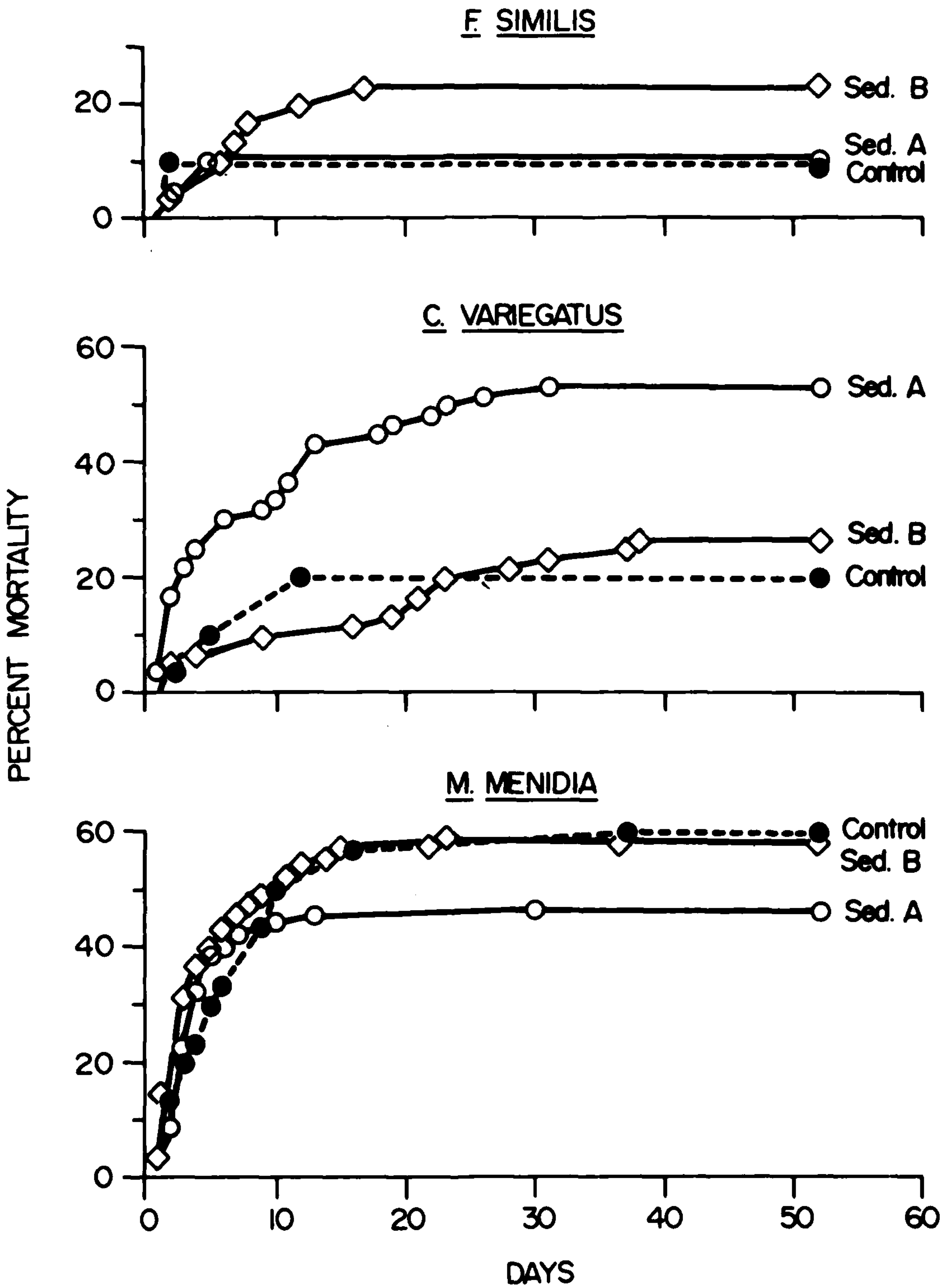

Figure 3. Mortality of test species exposed to sediments A and B and control sand for 52 days

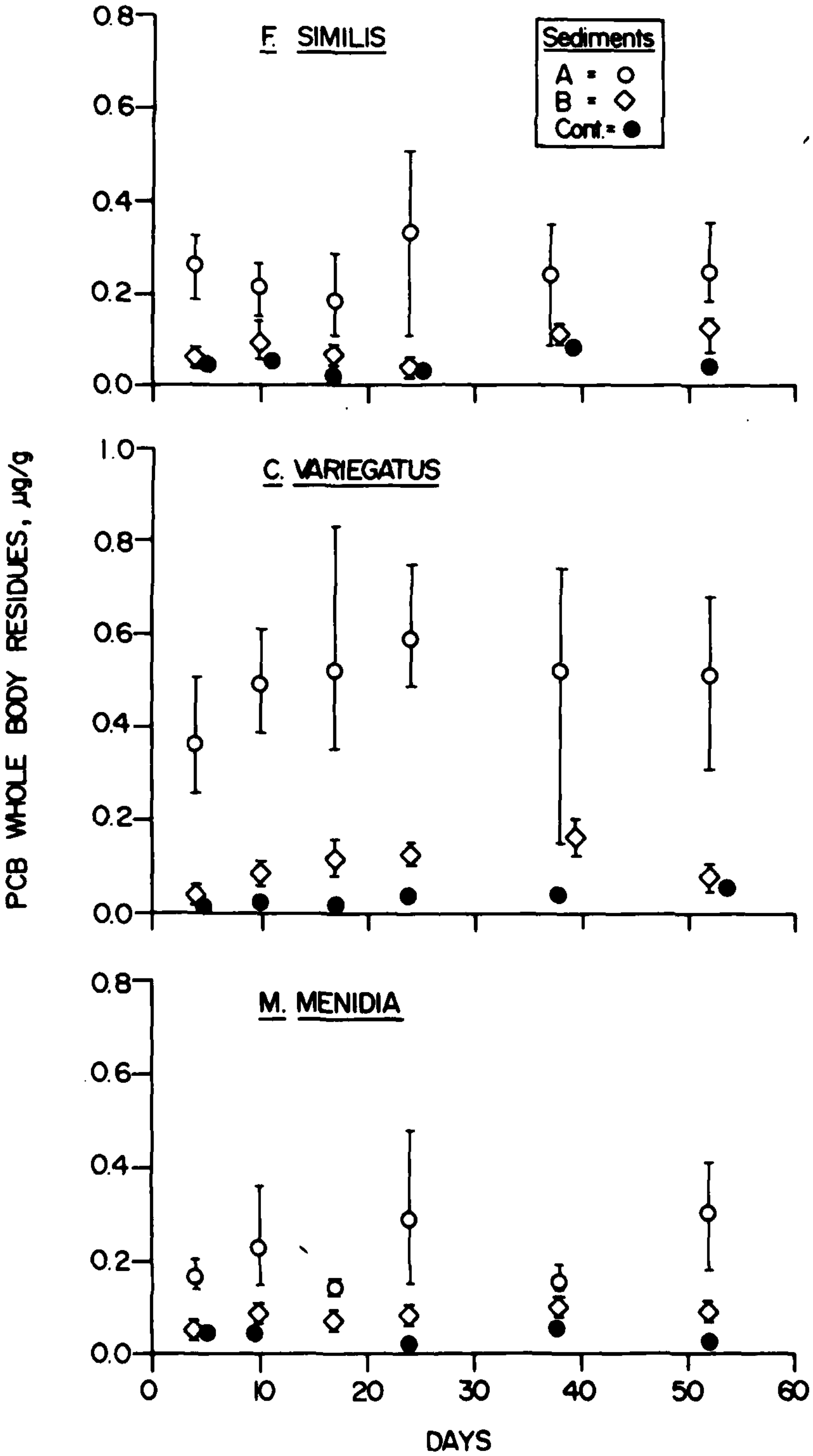

Figure 4. PCB whole body residues (wet weight) in test species through 52 days of exposure to test sediments (N = 3) and control sand

21. Cyprinodon also showed a statistically significant acute response (53 percent mortality) to sediment A. Although some mortality (25 percent) was observed in sediment B, it was not distinguishable statistically from the control (20 percent). Fundulus displayed a lower order of acute response to test sediments. Mortality in sediment B was statistically different from the controls, but sediment A did not differ from controls. The Menidia control mortality was high enough that statistical analysis was not warranted.

Bioaccumulation

22. For each of the three species, significant differences ($p < 0.05$; Figure 4; Tables 2 and 3) in PCB whole body residues (wet weight) among sediment types were detected using analysis of variance (SAS 1982). Duncan's multiple comparison procedure, applied after each analysis, showed that the mean PCB whole body residue for sediment A was significantly greater than the mean residue for the control sediment; however, there was no statistically significant difference between sediment B and the control sediment. Highest PCB body burdens were found in C. variegatus (Table 3). Maximum average PCB concentration measured in C. variegatus exposed to sediment A was 0.59 µg/g wet weight compared to 0.33 µg/g in F. similus and 0.29 in M. menidia.

23. PCB uptake from sediment A was rapid; by day 4, C. variegatus accumulated 62 percent, F. similis accumulated 80 percent, and M. menidia accumulated 58 percent of the maximum body burden measured during the 52-day exposure. This is in contrast to the more gradual rate of PCB accumulation measured in infaunal polychaetes exposed to contaminated sediments (Fowler et al. 1978; Elder, Fowler, and Polikarpov 1979;

Table 2. Analysis of Variance Tables for Whole body Residues by Species

Source of Variation	d.f.	Sum of Squares	F-Value	Pr > F
		F. similis		
Sediment type	2	0.2807	17.00	<0.05
Time	5	0.0147	0.36	0.87
Sediment type X time	10	0.0378	0.46	0.90
Error	21	0.1733		
		C. variegatus		
Sediment type	2	1.7504	38.39	<0.05
Time	5	0.0438	0.38	0.85
Sediment type X time	10	0.0302	0.13	0.13
Error	23	0.5244		
		M. menidia		
Sediment type	2	0.1724	15.04	<0.05
Time	5	0.0077	0.27	0.92
Sediment type X time	10	0.0569	0.99	0.48
Error	21	0.1204		

Table 3. PCB Whole Body Residues (μg/g, wet weight) for *C. variegatus*, *F. similis*, and *M. menidia* Exposed to Sediment A and B and Control Sand

Sediment Type	day	0	4	10	17	24	38	52
				C. variegatus				
Sediment A		0.046	0.504	0.477	0.828	0.532	0.660	0.309
		0.053	0.259	0.610	0.381	0.747	0.150	0.545
		0.055	0.323	0.388	0.353	0.489	0.738	-
	$\bar{x}$	0.051	0.362	0.492	0.520	0.589	0.516	0.427
	S.D.	0.003	0.103	0.091	0.218	0.113	0.261	0.118
Sediment B			0.045	0.109	0.084	0.119	0.128	0.105
			0.044	0.096	0.102	0.109	0.202	0.088
			0.018	0.057	0.161	0.147	-	0.046
	$\bar{x}$		0.036	0.087	0.116	0.125	0.165	0.079
	S.D.		0.012	0.022	0.033	0.016	0.037	0.025
Control			0.012	0.020	0.020	0.030	0.042	0.070
				F. similis				
Sediment A		0.055	0.323	0.142	0.286	0.506	0.104	0.188
		0.022	0.277	0.262	0.137	0.109	0.273	0.355
		0.017	0.187	0.243	0.137	0.381	0.349	0.205
	$\bar{x}$	0.031	0.262	0.216	0.187	0.332	0.242	0.249
	S.D.	0.017	0.056	0.053	0.070	0.166	0.103	0.075
Sediment B			0.045	0.153	0.046	0.045	0.093	0.073
			0.088	0.060	0.048	0.044	0.133	-
			0.055	0.069	0.112	0.036	-	-
	$\bar{x}$		0.060	0.094	0.069	0.042	0.113	-
	S.D.		0.015	0.042	0.031	0.004	0.020	-
Control			0.089	0.062	0.021	0.048	0.137	0.051
				M. menidia				
Sediment A		0.073	0.151	0.153	0.138	0.232	0.195	0.412
		0.065	0.206	0.362	0.152	0.480	0.104	0.180
		0.056	0.149	0.173	-	0.159	0.163	0.318
	$\bar{x}$	0.064	0.169	0.229	0.145	0.290	0.154	0.303
	S.D.	0.007	0.026	0.194	0.007	0.137	0.038	0.095
Sediment B			0.045	0.088	0.084	0.066	0.120	0.092
			0.053	0.112	0.074	0.110	0.085	-
			0.061	0.066	0.059	0.082	0.080	-
	$\bar{x}$		0.053	0.087	0.072	0.086	0.095	-
	S.D.		0.006	0.019	0.010	0.018	0.018	-
Control			0.070	0.074	-	0.020	0.055	0.023

Rubinstein, Lores, and Gregory 1983) and suggests that uptake by these fishes is primarily a function of PCB equilibrium-partitioning from water. Califano, O'Connor, and Peters (1980) also reported rapid rates of PCB accumulation from water by striped bass (Morone saxatilis).

24. Variability in PCB body burden between individuals and species was apparent (Figure 4; Table 3) and is related to differences in lipid content of the organisms (Philips 1980). Major factors that affect the lipid content and, consequently, body burden of hydrophobic compounds are: age or size, diet, state of health, and reproductive cycle. The most consistent PCB residue data were observed in C. variegatus; greater fluctuations were measured in M. menidia and F. similus. A possible explanation for the variability observed in M. menidia is related to the 2-week spawning cycle displayed by this species. During spawning, the organisms released considerable amounts of lipid and associated lipophilic compounds such as PCBs. Unfortunately, due to limited sample size, we could not normalize PCB residues on the basis of lipid content. However, we are able to report pooled lipid values for each species. Lipid content (by the method of Bligh and Dyer (1959)), based on dry weights, measured 4.8 percent for C. variegatus, 1.8 percent for F. similus, and 6.8 percent for M. menidia.

Recommendations and Conclusions

25. On the basis of species selection criteria and past studies, all three fish species appear suitable as bioassay organisms and have been so used in development of ambient water quality criteria. However, on the basis of comparative LC_{50} values, M. menidia has been shown to be the

most sensitive of the three species examined (EPA 1980; Roberts et al. 1982; Middaugh and Dean 1977). Although acute sensitivity serves as a strong endorsement for species selection, other factors must also be considered (i.e., species availability, ease of handling and shipping, etc.) The toxicity data generated in this study showed excessive control mortality (> 10 percent); therefore, a definitive statement regarding comparative acute sensitivity cannot be made. It is apparent, however, from this study that potential problems regarding potentially very high sediment resuspension caused by swimming activity must be considered in the exposure design of solid phase bioassays. Halter and Johnson (1977) showed that fathead minnows (Pimephales promelas) in direct contact with sediments accumulated PCB residues at six times the rate of those screened from direct contact with sediments. If fish are to be used for solid phase testing, exposure concentrations of suspended solids should be simulative of the disposal sites since suspended solids concentration greatly influences bioavailability of organic contaminants (Karickhoff, Brown, and Scott 1979).

26. Fish appear to be excellent indicators of contaminant bioavailablity. Seelye, Hesselberg, and Mac (1982) showed that fish can accumulate several common environmental contaminants, including PCBs, directly from resuspended sediments. In our study, all three species accumulated PCBs from sediments in a dose-dependent manner. Most uptake was observed within the 10-day exposure period recommended for solid phase bioassays. Cyprinodon variegatus appeared to accumulate more PCBs from sediment A than the other species; however, body burdens were not normalized for lipids. Whenever possible, residue data for hydrophobic compounds should be based on lipid content rather than wet weight (Schneider 1982).

27. Although we encountered shipping and handling problems with adult *Menidia*, we believe that these problems can be overcome by culturing them in the laboratory (Middaugh 1981) or by shipping eggs or juveniles. If these techniques prove reliable, *Menidia* is the species of choice for solid phase evaluations of dredged material emphasizing both toxicity and bioaccumulation, and would serve as a suitable substitute for species currently being tested. If the concern is primarily with bioaccumulation, *Cyprinodon* may be more suitable due to its hardiness and consistency in measured whole body PCB residues.

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